AD_____

Award Number:
W81XWH-08-1-0202

TITLE:
Post-Stress Combined Administration of Beta-Receptor and Glucocorticoid Antagonists as a Novel Preventive Treatment in an Animal Model of PTSD

PRINCIPAL INVESTIGATOR:
David Morilak, Ph.D.

CONTRACTING ORGANIZATION:
University of Texas Health Science Center
San Antonio, TX 78229

REPORT DATE:
July 2009

TYPE OF REPORT:
Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

☑ Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01-07-2009	Annual	1 July 2008 – 30 June 2009

4. TITLE AND SUBTITLE

Post-Stress Combined Administration of Beta-Receptor and Glucocorticoid Antagonists as a Novel Preventive Treatment in an Animal Model of PTSD

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-08-1-0202

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

David Morilak, Ph.D., Principal Investigator

Email: morilak@uthscsa.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

AND ADDRESS(ES)

University of Texas Health
Science Center

San Antonio, TX 78229

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND

FORT DETRICK, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Some PTSD symptoms, e.g., social withdrawal, generalized anxiety and stress-sensitization, may reflect pathologically enhanced memory of traumatic stress. Stress-induced secretion of brain norepinephrine and glucocorticoids (GC) activate β-receptors and GC-receptors in the amygdala, enhancing consolidation of emotional memories. Thus, giving a β-antagonist plus a GC-antagonist immediately after stress might decrease the strength of those associations, and prevent the emergence of PTSD. The goals of this work were to 1) validate Massed Footshock (MFS), in which rats are exposed to a single session of repeated footshock, as a model of PTSD; 2) establish a reliable test battery of PTSD-like behaviors in rats; and 3) test the combined drug treatment after MFS. We established a reliable test battery for PTSD-like behavior, and drug treatment did not interfere with testing, nor induce non-specific effects. However, MFS proved to be neither a valid nor useful model of PTSD. It failed to affect the most relevant behavioral measures, and confounded fear conditioning. Thus, for the remainder of this project, we plan instead to employ a modified Single Prolonged Stress (SPS) model, that has been reported to elicit relevant behavioral effects and retains the temporal features of MFS that made it amenable to acute pharmacological intervention.

15. SUBJECT TERMS
Rats, massed footshock, PTSD, open field test, social interaction test, fear conditioning, extinction, beta-adrenergic receptors, glucocorticoid receptors, propranolol, mifepristone

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	13	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

Introduction…….. 4

Body…….. 4-11

Key Research Accomplishments…….. 11

Reportable Outcomes…….. 12

Conclusion and Plans…….. 12-13

References…….. 13

Appendices…….. none

Project W81XWH-08-1-0202 (CDMRP PTSD Concept Award PT073760)
Post-Stress Combined Administration of Beta-Receptor and Glucocorticoid Antagonists as a Novel Preventive Treatment in an Animal Model of PTSD
Annual Progress Report

A. Introduction

The hallmark symptoms of PTSD – social withdrawal, re-experiencing, generalized anxiety and stress-sensitization, may all be viewed as manifestations of a pathologically enhanced and persistent memory of the trauma. The immediate response to stress is characterized by sympathoadrenal activation, with secretion of glucocorticoid hormones and plasma catecholamines, and release of the neurotransmitter norepinephrine (NE) throughout the brain, facilitating and amplifying physiological, behavioral, emotional and cognitive responses to the stress. These systems all converge in the limbic forebrain, in particular in the amygdala, where NE and glucocorticoids activate β-adrenergic receptors and glucocorticoid receptors that synergize to enhance anxiety and fear, and strengthen the consolidation of emotional memories for stressful events in the immediate post-stress period. We thus hypothesized that treatment with a combination of a β-adrenergic antagonist plus a glucocorticoid antagonist immediately after a traumatic experience might decrease the strength of the abnormally enhanced associations, and prevent the emergence of PTSD-like behavioral symptoms over time. Our proposal was to test this hypothesis in an animal model of PTSD, using a battery of behavioral tests to assess key PTSD-like symptoms in rats, including social withdrawal on the social interaction test, generalized anxiety and stress-sensitization on the elevated plus-maze, enhanced fear conditioning and reduced extinction of cue-conditioned fear. The temporal characteristics of the model must be amenable to testing acute drug treatment in the immediate post-stress period, so the model we chose from the literature was the Massed Footshock (MFS) model, in which rats are exposed to a single session of repeated footshocks. This model has been reported to produce delayed, long-lasting changes in behavior and physiology that are reminiscent of PTSD (Stam, 2007). We could then administer a combination of the β-receptor antagonist propranolol and the glucocorticoid antagonist mifepristone immediately after the MFS treatment. Our original Statement of Work comprised 3 experimental tasks – first to validate MFS as a model of PTSD and establish a reliable and relevant behavioral test battery, then to test the drug treatment regimen given either before or after MFS.

B. Body

The bulk of our efforts to date have been aimed primarily at addressing Task 1 in the Statement of Work, to establish a valid model of time-dependent emergence of PTSD-like behavioral symptoms after exposure to a single session of massed footshock exposure.

Validation of this model is of course essential to be able to then use it to study efficacy of a novel pharmacologic intervention given either before (Task 2) or more importantly and more relevant to PTSD, after stress exposure (Task 3). The massed footshock model was adapted from a literature published primarily by a single research group (reviewed in Stam, 2007). However, we felt that we had a good starting place, in that we had already seen a delayed effect on some of the relevant behavioral measures to be used in this project in previous studies we had done using a chronic unpredictable stress treatment. Although the chronic protocol was not amenable to testing the novel acute pharmacologic intervention we proposed in this concept award project, it nonetheless gave us reason to think that at least some of the key behavioral measures would be sensitive to time-dependent effects of acute massed footshock.

Rather than employ all of the behavioral tests outlined in steps 1-5 in the initial pilot studies, we decided to use only those that were most relevant to PTSD and/or most easily

implemented, allowing us the flexibility to test various time points (hence the elimination, for these first studies, of the cognitive set-shifting test, which requires 1 week of food restriction prior to testing, and thus locks it into a fixed testing day). To begin, we used the open-field test (OFT), a measure of general arousal, the social interaction (SI) test, a measure of social behavior, and the elevated plus-maze (EPM), a measure of generalized anxiety.

The second major focus of our initial studies has been to set up and validate the fear-conditioning and extinction test protocol in our lab. Not only was this seen as the most crucial test to assess major behavioral components of PTSD (hyper-sensitization and generalization of conditioned fear, and failure to extinguish aversive memories), but it was the only test proposed that we had not yet established in our lab. These two major emphases are described below.

B.1. Test time course of behavioral effects after a single massed footshock session

Work began on this project in the late summer and fall of 2008 with a series of pilot studies to examine whether, and over what time frame, a single session of massed footshock (MFS) exposure would evoke a reliable, reproducible and significant behavioral effect detectable on the OFT, SI, and EPM tests. Forty-two adult male Sprague-Dawley rats were assigned to three groups (n=14 per group). On day 0, two group were exposed to a single session of MFS (10 x 5 sec scrambled footshocks, 1.25 mA, delivered at varying intervals for 15 min), and the remaining group served as unstressed controls. Rats from all three groups were then tested over 4 days, beginning at either 7 or 14 days after MFS exposure. They were first tested in the OFT, which also served as habituation to the SI arena. After 2 additional habituation days, including 2 min of handling, they were then tested on the SI and EPM on the 4th day of testing. In addition, to determine whether a single session of MFS would sensitize the response to a subsequent acute stressor, 5 min of acute immobilization stress was administered to a subset of rats in each condition 20 min before testing on the SI and EPM.

The only significant behavioral effect observed after the single MFS treatment was a decrease in locomotor activity on the Open Field test at 7 days, but not 14 days post-MFS (Figure 1) Importantly, there were no effects on the EPM (data not shown), nor on the Social Interaction test, the most essential measure of the three for modeling a dimension of PTSD. Thus, we were unable to replicate the long-lasting changes reported after MFS (Stam 2007).

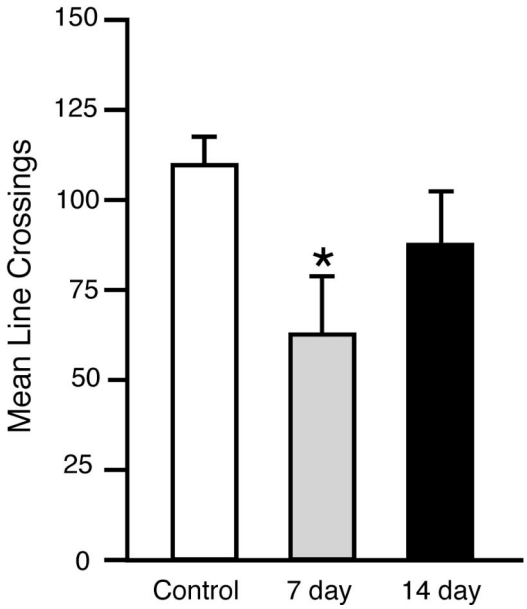

Figure 1. Rats exposed to a single MFS session exhibited reduced locomotion in the open field test 7 days after the MFS session (*p < 0.05 compared to controls). By 14 days, locomotion in the OFT had returned to control levels. Data expressed as mean number of line crossings in 5 min (mean ± SEM, n = 14 per group).

B.2. Second study – Modifications to enhance the potential impact of the MFS procedure

Given the minimal effect of single MFS exposure on behavior only on the OFT, and the absence of any effect on the more critical measures for modeling components of PTSD, we tried some modifications to the MFS procedure in an attempt to enhance the impact of the massed footshock, while at the same time retaining the acute nature of the stimulus, so as to still be able to test the efficacy of a novel acute pharmacological intervention as originally proposed. Thus, in the next study, instead of a single acute MFS exposure, we employed three MFS sessions. The initial session was followed by two identical reminder sessions, each separated by one week. In addition, and again to maximize the impact of the stressors, we reduced the handling time of the rats prior to testing. Thirty rats were assigned to the MFS (n=20) or control (n=10) conditions (two control groups of n=10 were used, one exposed to the chambers, one not. As there was no difference, these control groups were pooled). Rats were handled for only 1 min on each treatment day, then placed in the SI arena for 5 min prior to the MFS or control session. As described in Task 1 of the SOW, the purpose was to associate the benign SI context with the stress. MFS was then administered as described above. Controls were placed in the chamber but no shock was applied. Rats were allowed 1 hr recovery after the MFS or control treatment before returning them to housing. This procedure was repeated three times, separated by 1 week each. On day 3 following the final treatment session, rats were tested on the OFT. The following day (day 4), they were tested on the SI test followed immediately by the EPM. Time course was not tested in this pilot; the main purpose was simply to get effects on EPM and SI.

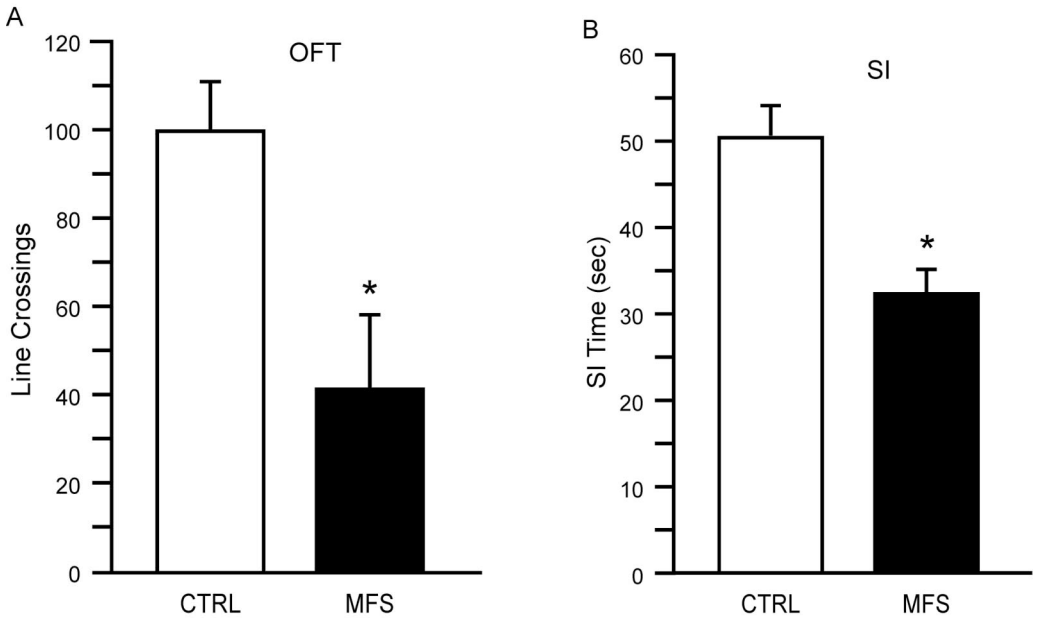

Figure 2. Rats tested 4 days after the last of three MFS sessions exhibited reduced locomotion in the open field test (A) and reduced social behavior in the SI test (B), *p < 0.01 compared to controls; mean ± SEM, n = 10-20 per group for OFT, n=5-10 per group for SI, as half in each group were exposed to immobilization stress, with no MFS effect observed).

Results indicated only a partial improvement. The effect on exploratory behavior in the OFT was replicated ($F_{1, 28} = 9.365$, $p < 0.01$; Figure 2A), and in this experiment, there was also a significant reduction in Social Interaction ($F_{1,28} = 10.638$, $p < 0.01$; Figure 2B), but no significant

effect on open-arm exploration in the elevated plus-maze (not shown). This is crucial, as we have shown repeatedly that 4 days after the end of another stress procedure, the chronic unpredictable stress protocol, there is a reliable and robust decrease in open-arm exploration on the EPM, a measure of state anxiety (Bondi et al., 2008). That is in fact why we focused on the 4-day time point in this study. In addition, as per Task 1 of the SOW, half the rats in each group were exposed to acute immobilization stress prior to SI testing, but no MFS effect was observed (not shown). Nonetheless, the decrease in social interaction was promising, although even this proved not to be replicable or consistent in subsequent experiments (see section B.4 below).

B.3. Replication and refinement of the 3-MFS procedure before drug treatment

Twelve rats were assigned to the 3-MFS or control conditions. To avoid any potential confounds of testing EPM and SI on the same day, SI and EPM testing took place on days 3 and 4 after the last MFS session, respectively (although we had previously validated sequential testing on the same day with no confounds, that was not done after a stress pre-treatment). To minimize animal use, we eliminated the non-chamber-exposed control group, as there were no differences in the preceding study. We also eliminated the acute immobilization stress prior to either SI or EPM testing until a reliable and replicable effect could be obtained on the basal behavioral parameters on both of these tests. Thus, in this pilot, OFT was tested on day 3, SI on day 4, and EPM on day 5 following the last of 3 weekly MFS sessions. Finally, rats were handled only during the MFS or control sessions, with no additional handling before testing.

As in the previous study, there was a significant effect of the 3-MFS treatment on locomotion in the OFT and social interaction in the SI test (Control: 61 $\pm$ 7 sec, MFS: 29 $\pm$ 5 sec; mean $\pm$ SEM; $p < 0.01$). But once again there was no effect on open-arm exploration in the EPM. Thus, in this study, we replicated the effects of the 3-MFS treatment on the OFT and SI tests, although we continued to be unsatisfied with the lack of effect on the EPM, and the lack of any enhancement of the effects of acute immobilization stress on SI or EPM behavior (**cf. steps 1, 2, 4, and 5 of Task 1 in the SOW**). And we had not shown an extended duration of effect. Nonetheless, this limited success offered sufficient justification to try an initial test of the drug treatment regimen originally proposed, but with the following modifications: an elaboration of the stress treatment to include 3 weekly MFS sessions; a compressed time frame for testing, to 3-5 days after the last MFS session; and a reduced behavioral test battery including OFT, SI and EPM, but excluding the time- and labor-intensive cognitive set-shifting test (**step 3 of Task 1 in the SOW**), and excluding the fear conditioning and extinction (**steps 6-7 of Task 1 in the SOW**), that were still under development in the lab at that time (see section B.5. below).

B.4. Combined treatment with a β-adrenergic receptor antagonist, propranolol, and a glucocorticoid receptor antagonist, mifepristone, immediately after each MFS session

In this first test of the combined drug intervention intended to attenuate consolidation of the aversive consequences of MFS exposure, we focused on giving the drug treatment immediately after the MFS exposure, as proposed in **Task 3 of the SOW**. This post-stress treatment is not only the most relevant to the consolidation process, but would also be the most likely mode of treatment in the field, i.e., administered after exposure to a traumatic event.

Thirty-seven rats were assigned to MFS or control groups. The MFS treatment was modified as above to be given in 3 weekly sessions. Immediately after each MFS or control session, rats received injections of propranolol (10 mg/kg in a volume of 3 ml/kg, i.p.) and mifepristone (25 mg/kg in a volume of 1 ml/kg, i.p.) or comparable injections of saline vehicle.

Rats were returned to the housing facility 1 hr after injections. Behavioral testing took place, as above, on day 3 (OFT), day 4 (SI) and day 5 (EPM) after the last MFS or control session.

The results of this study, conducted using two independent cohorts of rats, were unfortunately inconclusive. While the reduction in exploration on the OFT was again replicated (Figure 3A), this time there was no significant effect of the 3-MFS treatment on social interaction (Figure 3B). And again, as above, there was no effect on EPM. Moreover, there was no effect of drug treatment on any measure (Figure 3). However, given the lack of MFS effect on the two most critical behavioral measures (SI and EPM), the lack of drug effect was uninformative. Thus, the primary conclusion to be reached from this series of studies is that the single massed footshock procedure is an unreplicable and unreliable model of lasting behavioral changes of any relevance to PTSD. Increasing the treatment to 3 MFS sessions was only marginally better than single MFS, eliciting an inconsistent reduction in social behavior as well as the decrease in open field exploration. Thus, we plan in future to adopt a new stress model (see E, below).

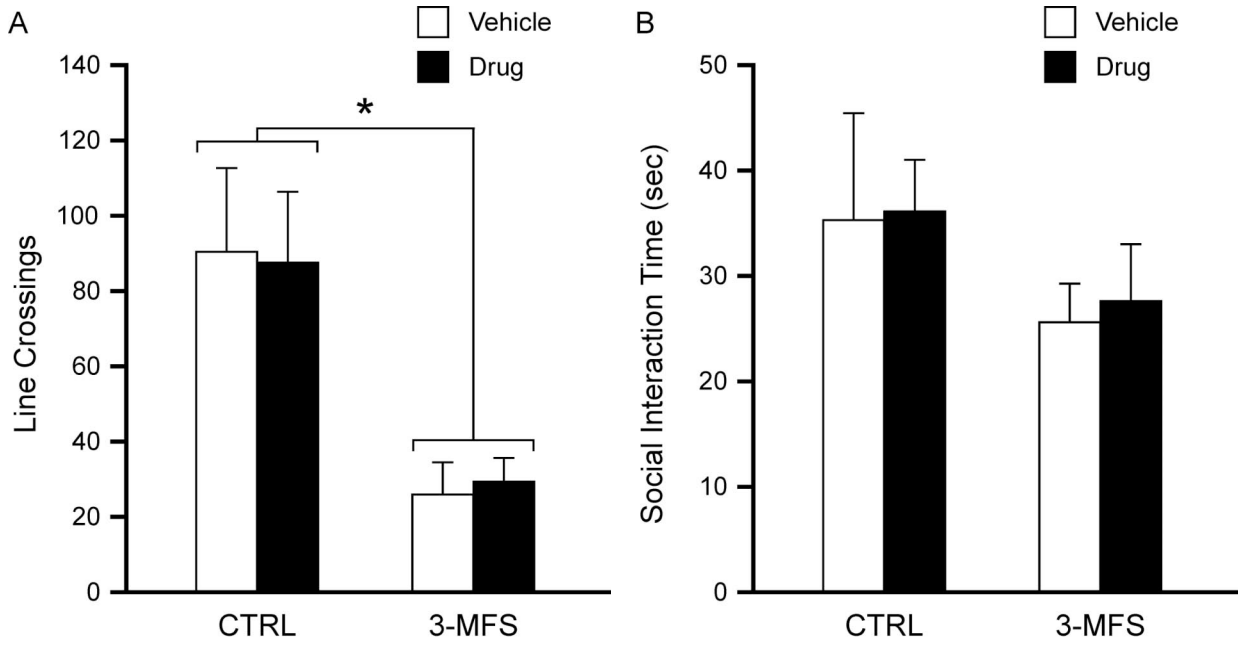

Figure 3. Rats tested 4 days after the last of three MFS sessions exhibited reduced locomotion in the open field test (A) but in this study, social interaction was unaffected (B). Combined treatment with the β-receptor antagonist, propranolol (10 mg/kg) and glucocorticoid-receptor antagonist, mifepristone (25 mg/kg) given immediately after each MFS or control session, had no effect on baseline behavior in either test, nor on the reduction in OFT behavior seen after MFS exposure. *$p < 0.01$ compared to controls; mean ± SEM, n = 7-9 per group.

B.5. Development of a procedure for assessing fear conditioning and extinction

A second critical dimension of PTSD is sensitization of fear-conditioning and failure to show extinction of conditioned fear. This results in heightened expression of a learned fear response to appropriate stimuli associated with the traumatic stress and also to inappropriate stimuli unrelated to the trauma, and a failure to exhibit appropriate extinction of the conditioned fear response upon returning to a non-threatening environment. This was the only behavioral

test procedure in the proposal not yet established in our lab. Thus, this year we purchased new equipment for conducting fear conditioning and extinction from a different funding source, as this technique will be used more generally in our research program, but it will be applied most immediately to this project. Thus, we have spent considerable time and effort establishing the optimal parameters and test conditions for generating a reliable conditioned-fear response, and a reliable process of extinction (**steps 6 and 7 in Task 1 of the SOW**).

For these pilot studies to develop the optimal fear conditioning and extinction procedure, control rats from previous studies that had not been exposed to any kind of stress were used.

General Procedure

A new fear conditioning apparatus and control system from Coullbourn Instruments was used (model # H10-11R-TC), with two shock chambers. In order to explicitly assess cue-conditioning and to avoid contextual conditioning to the test chamber itself, a "Context A" was used for conditioning, and a "Context B" was used for all subsequent testing and extinction sessions. Context A was simply the unmodified, rectangular shock chamber, with reflective metal walls and a metal grid floor. Context B was modified by placing a flat, green vinyl mat on the floor over the grids, and wrapping a thin, flexible black-and-white vinyl mat around the interior, forming a circular chamber wall with color, odor and texture different from those in the training context. The chambers were enclosed in sound-attenuating booths. Rats were habituated to both contexts for 15 min, in counterbalanced order, the day before conditioning.

On day 1 (24 hrs. after habituation), cued fear conditioning was conducted in Context A. After 5 min acclimation, rats were presented with 2 pairings of a 20 sec tone (10 kHz, 75 dB) that co-terminated with a footshock (0.5 sec, 0.7 mA) delivered through the grid floor. The inter-trial interval (ITI) was 120 sec. These parameters were selected to produce approximately 50% freezing during the first retention test in naïve rats (Burghardt et al., 2004, 2007). On day 2, rats were tested for retention in context B. The dependent measure was freezing behavior in response to presentation of the tone alone, with no shock delivered. This also constituted the first extinction trial, with 3 extinction trials (ITI 90-120 sec.) presented on each of days 3-5.

Freezing was recorded and analyzed using FreezeFrame and FreezeView software from Coulbourn. Freezing was defined from an activity histogram that plotted the number of video frames on the y-axis and the motion index value (a measure of the number of pixels that changed from one frame to the next) plotted on the x-axis (Figure 4). A freezing threshold was determined for each rat from activity recorded over 4 min at the end of the habituation period in Context A. Freezing was first measured during the 20 sec prior to tone 1 on day 1, then during each tone presented thereafter, and expressed as a percentage (*m*) of the 20 sec period.

B.5.1 Fear-conditioning and extinction in naïve rats

Male Sprague Dawley rats (n=5), 350-390g, were singly housed, and handled for 1 min. on 3 consecutive days before fear conditioning and extinction were conducted as described above. On day 1, rats displayed little freezing during the pre-tone baseline (*m* = 0%) or during the initial presentation of tone 1 (*m* = 1.89%). After the first tone-shock pairing, rats displayed increased freezing to tone 2 (*m* = 12.43%), reflecting acquisition and short-term retention of conditioned fear. They displayed even greater freezing to tone 1 on day 2 (*m* = 31.62%), indicating consolidation and retention. During extinction, the rats initially increased freezing to each tone presented on day 2 (*m* = 38.11% and 67.84% to tones 2 and 3, respectively), but on subsequent days, they displayed a decrease in freezing until they reached relatively low levels in response to the last tone on day 5 (*m* = 13.51%). To score extinction over days, a mean response to the three tones presented on each day was calculated, revealing a steady decline

in freezing behavior from 45.86% on day 2 to 17.35% on day 5 (Figure 5), consistent with results reported by other groups using a similar protocol (Burghardt et al., 2004, 2007).

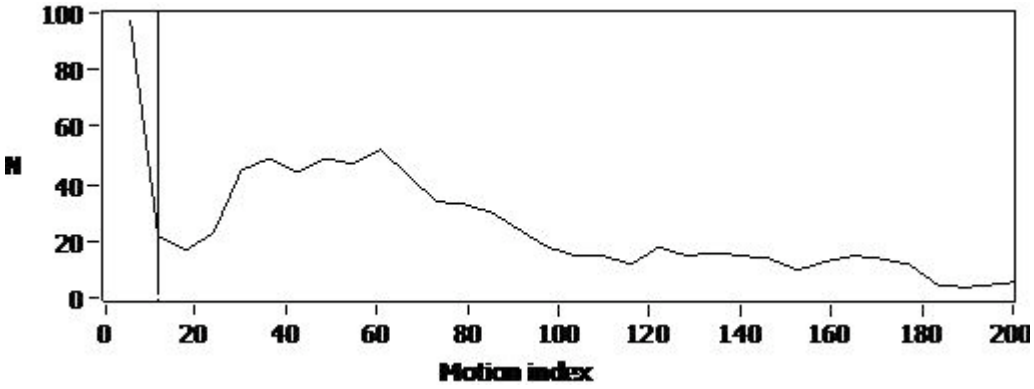

Figure 4. A typical motion index histogram collected for the fear-conditioning test. Behavior in the shock chamber was recorded and captured electronically. The y-axis indicates the frequency with which a video frame showed a given motion index value, plotted on the x-axis, reflecting the change in pixels relative to the preceding frame. The freezing threshold, indicated by the vertical line, was defined by the point of maximum deflection between the peak of low-motion range scores, and the peak of high motion range scores, in this case at a motion index value of ~12.0.

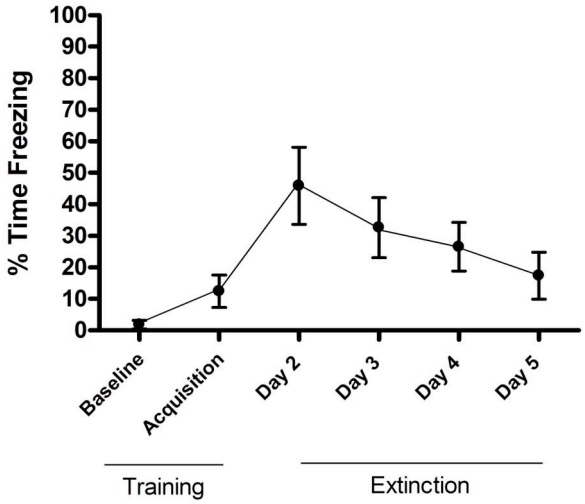

Figure 5. Freezing behavior across days, showing low baseline freezing, an increase in freezing in response to the tone with acquisition of cued fear conditioning, a greater increase to near 50% freezing with consolidation and retention on day 2, followed by a gradual decrease with extinction over days 2-5 (mean ± SEM, n=5).

B.5.2 Effects of massed footshock on fear conditioning and extinction

In this next experiment, 9 rats were singly housed and handled for 1 min on 3 consecutive days. The next day, they were exposed to a single MFS (n=5) or control (n=4) session. Five days later, fear conditioning and extinction were conducted as described above.

Overall, the pattern of behavioral response was similar to that seen in experiment 5.1. Both groups showed little freezing during the pre-tone baseline (*m* = 2.4% and 0% for MFS and

controls, respectively) and during the first tone (*m* = 0% and 6.42%). Both groups also showed acquisition and increased freezing to tone 2. However, the MFS-stressed rats displayed *less* freezing overall than the non-stressed control rats (Figure 6). This difference was greatest on day 2 (*m* = 50.54% and 81.89% freezing for MFS and control groups, respectively), persisted on days 3 (*m* = 33.30% and 55.32%) and 4 (*m* = 30.8% and 57.7%), and both groups showed a similar level of extinction by day 5 (*m* = 29.65% and 32.93%). Thus, MFS exposure appeared, if anything, to impair fear-conditioning, an effect opposite in direction to the predicted effect.

This apparent impairment of fear-conditioning in the MFS-exposed rats was obviously an unexpected finding. However, upon examination of some older literature, we found evidence that in classical conditioning paradigms, pre-exposure to either the unconditioned stimulus (in this case, footshock) or to the conditioned stimulus (i.e., tone) can impair later associative conditioning (Randich and LoLordo, 1979). Based on this, we presume that because the MFS stress procedure constitutes a pre-exposure to footshock, it precludes the use of footshock as a US in the fear conditioning procedure. Thus, in addition to the limited and inconsistent behavioral effects obtained on the SI and EPM tests following MFS (see above), this confound also makes MFS an unsuitable model for this project for more practical reasons as well.

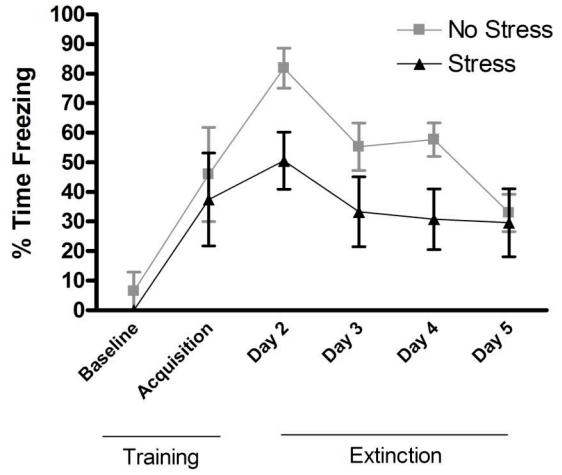

Figure 6. Effects of a single MFS exposure on fear conditioning and extinction. Both MFS and control rats showed low baseline freezing, and both groups exhibited an increase in freezing in response to the tone after it was paired with footshock. However, acquisition of fear-conditioning was impaired in the MFS-pre-treated rats (mean ± SEM; n=4-5 per group).

C. Key Research Accomplishments

-Established a reliable and effective behavioral test battery to assess key PTSD-like behavioral changes that emerge over time after a traumatic experience in rats

-Established a reliable and effective test that is sensitive to both enhanced and attenuated fear conditioning and extinction

-Established a drug treatment regimen that is feasible in the proposed paradigm, does not interfere with behavioral testing, and does not elicit non-specific effects in control animals

-Tested extensively the MFS model and the 3-MFS variant thereof, and concluded that it is neither a valid nor useful model of relevance to PTSD

-Formulated a viable plan to replace MFS with a modified SPS model, retaining the key characteristics that make it amenable to the planned behavioral tests and drug intervention

D. Reportable Outcomes

1. Meeting abstract – for both a poster presentation and a talk:

Morilak, DA, Joshi, A, Rodriguez, G (2009) Developing a rat model of delayed behavioral stress reactivity in PTSD suitable to investigate potential pharmacologic interventions. Congressionally Directed Medical Research Programs Military Health Research Forum, Kansas City, MO, Aug 31-Sept 3, 2009.

2. Meeting abstract – for poster presentation only (archival abstracts, to be published in 09/09):

Joshi, A, Rodriguez, RA, Morilak DA (2009) Delayed stress reactivity after footshock: A rat model for PTSD. Soc Neurosci Abstr 35 Online Program, in press.

E. Conclusions and plans

We have made substantive progress in establishing an effective time frame and optimal procedures for conducting a thorough and valid behavioral test battery to assess several of the most relevant and essential behavioral dimensions of PTSD following cessation of stress: day 3 for open field, day 4 for SI, day 5 for EPM, day 6 for fear-conditioning, days 7-9 for extinction. We have also established a procedure for acute systemic administration of the two drugs of interest in this project immediately after exposure to an acute traumatic stressor that does not interfere with subsequent testing, nor induce non-specific effects in controls.

We have also made substantive progress specifically in establishing a fear-conditioning and extinction procedure that elicits a level of freezing that is sensitive to detect both enhanced as well as attenuated conditioning, and a trajectory for extinction that will do likewise.

However, we have made an extensive and earnest effort to establish and confirm MFS as a valid and useful model of PTSD for this project, and we are now convinced that it is neither valid nor useful. It failed to affect the key behavioral measures of most relevance to PTSD, and it presents an unexpected confound for carrying out the fear conditioning and extinction tests. Thus, for the remainder of this project, we now plan to employ another model of PTSD, an adaptation of the Single Prolonged Stress (SPS) model that has been reported in the literature to elicit relevant changes in behavior (Khan and Liberzon, 2004; Yamamoto et al., 2008; Wang et al., 2008) and also retains the temporal features of the MFS model that make it amenable to testing an acute pharmacological intervention given at the time of the traumatic stress exposure.

The original SPS model involves sequential application of 3 stressors in a single session: immobilization, forced swim, and then anesthesia with ether, followed by exposure to a single conditioned shock context (e.g., Wang et al., 2008). However, because fear conditioning is essential to any model of PTSD, and as we have now shown that shock pre-exposure precludes fear conditioning to a footshock, we will modify this SPS procedure by using another stressor, social defeat stress, with which we have extensive experience in the chronic unpredictable stress model (Bondi et al., 2008), in place of the anesthesia plus footshock.

Thus, the modified SPS procedure that we will test to replace MFS in our project will entail:

1) 30 min of immobilization stress, followed immediately by

2) 20 min of social defeat (i.e., a single social defeat encounter followed by 20 min of continued exposure to the aggressor rat under a protective wire mesh cage to prevent further physical contact, but allowing sustained visual and olfactory interaction), followed immediately by

3) 10 min swim stress

This will thus keep the total duration of the entire stress exposure to 1 hour, consistent with our original proposal. We have extensive experience with all of the individual stimuli to be used in this sequence, in the course of our Chronic Unpredictable Stress model (Bondi et al., 2008), and all have been independently validated as robust psychogenic stressors. Once the SPS model has been validated using the key behavioral measures we now have in place, the acute drug administration procedure will then also be conducted exactly as proposed originally. Moreover, the full behavioral test battery that we have established will then be employed over the time frame described above, after drug intervention following the modified SPS treatment. We are in the process of obtaining IACUC approval for this change in procedure, and have contacted our Army Contracting Officer Representative to initiate the approval process for the change in SOW.

F. References

Bondi CO, Rodriguez G, Gould GG, Frazer A, Morilak DA (2008) Chronic unpredictable stress induces a cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment. *Neuropsychopharmacology* 33, 320-331.

Burghardt NS, Sullivan GM, McEwen BS, Gorman JM, LeDoux JE (2004). The selective serotonin reuptake inhibitor citalopram increases fear after acute treatment but reduces fear with chronic treatment: A comparison with tianeptine. *Biol Psychiatry* 55: 1171-1178.

Burghardt NS, Bush DEA, McEwen BS, LeDoux JE (2007). Acute selective serotonin reuptake inhibitors increase conditioned fear expression: Blockade with a 5-HT_{2C} receptor antagonist. *Biol Psychiatry* 62: 1111-1118.

Khan S, Liberzon I (2004). Topiramate attenuates exaggerated acoustic startle in an animal model of PTSD. *Psychopharmacology (Berl)* 172, 225-229.

Randich A, LoLordo VM (1979). Associative and nonassociative theories of the UCS preexposure phenomenon: Implications for Pavlovian conditioning. *Psychol Bull* 86: 523-548

Stam R (2007) PTSD and stress sensitisation: A tale of brain and body; Part 2: Animal models. *Neurosci Biobehav Revs* 31, 558-584

Wang W, Liu Y, Zheng H, Wang HN, et al. (2008). A modified single-prolonged stress model for post-traumatic stress disorder. *Neuroscience Letters* 441, 237-241.

Yamamoto S, Morinobu S, Fuchikami M, Kurata A, Kozuru T,Yamawaki S (2008). Effects of single prolonged stress and D-cycloserine on contextual fear extinction and hippocampal NMDA receptor expression in a rat model of PTSD. *Neuropsychopharmacology* 33, 2108-2116.